



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and
Agricultural Sciences

A pectic polysaccharide in seed gum of *Lepidium campestre*

En pektinrik gelsubstans extraherat ur frön från *Lepidium
campestre*

Anna Manneteg



Department of Molecular Sciences
Master's thesis • 30 hec • Second cycle, A2E
Agriculture Programme - Food Science
Molecular Sciences, 2017:17
Uppsala 2017

A pectic polysaccharide in seed gum of *Lepidium campestre*

En pektinrik gelsubstans extraherat ur frön från *Lepidium campestre*

Anna Manneteg

Supervisor: Roger Andersson, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Examiner: Galia Zamaratskaia, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Credits: 30 hec

Level: Second cycle, A2E

Course title: Independent project/degree project in Food Science - Master's thesis

Course code: EX0425

Programme/education: Agriculture Programme - Food Science

Place of publication: Uppsala

Year of publication: 2017

Cover picture: Licens Creativ Commons, Wikipedia; Anna Manneteg

Title of series: Molecular Sciences

Part number: 2017:17

Online publication: <http://stud.epsilon.slu.se>

Keywords: soluble dietary fiber, pectin, seed gum, hydrocolloid, field cress, *Lepidium campestre*, oilseed

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences

Abstract

Lepidium campestre (field cress) is an oilseed species with a great potential of becoming a new commercial crop in Sweden. As the oil is being extracted from the crop, the residual from the extraction is left in form of a seed cake. As this seed cake is soaked in water a gel is formed around the seeds. The purpose of this paper was to study the chemical composition of the seed gum extracted from *L. campestre* and to examine if a possible field of use exist for this substance. The seed gums function is tested, but no real rheological study is executed and no study is done on the seed itself. In this study, there is only one kind of seeds from one harvest analysed and different kinds of varieties within the species *L. campestre* are not studied.

The seed gum was separated from the seeds and freeze-dried before analysed. The determined molecular weight was 50-100 kDa, measured with Multi-Angle Light Scattering. The measured absorbance in light absorbance spectrum resulted in a detected substance at a wavelength of 280 nm, indicating aromatic amino acids in protein. This however was in a very small concentration. Determination of soluble dietary fibre was preformed according to The Uppsala Method, determination of neutral sugar residues by gas chromatography and for uronic acid by spectrophotometry. To further understand the structure of the seed gum, ¹H NMR spectroscopy was performed. The seed gum was consisting of up to 80 % dietary fibre and with a total yield of 7 % from weight seeds. The results showed that the seed gum consist of a high proportion of uronic acid, galactose and rhamnose - a rhamnogalactouronic pectic substance. The pectin contributes to a very stable and reliable seed gum that could be widely used in the food industry. Possible fields of applications are e. g. as dietary fibre-enrichment in food products or production of gluten-free bread which is tested in this study.

In this thesis, it is proven that it is a very easily extractable and easily isolated substance that does not have any smell and most likely no taste. From *L. campestre*, it is possible to get a substance made of a up to 80 % pure pectic substance, and all that is required is water and a mixer.

Keywords: soluble dietary fiber, pectin, seed gum, hydrocolloid, field cress, *Lepidium campestre*, oilseed

Sammanfattning

Lepidium campestre (fältkrassing) är en oljeväxt med stor potential att bli en ny kommersiell gröda i Sverige. När oljan extraheras från fröerna lämnas rester i form av en frökaka. När frökakan kommer i kontakt med vatten bildas en gel kring fröerna, en frö-gel. Syftet med denna uppsats var att studera den kemiska sammansättningen av den frö-gel som extraheras från *L. campestre* och undersöka om det finns ett möjligt användningsområde för substansen. Funktionen av frö-gelen testas, men ingen reell reologisk studie utförs och ingen undersökning görs på själva fröerna. I denna studie är det bara fröer från en skörd som analyseras och olika sorter inom arten *L. campestre* kommer inte att granskas.

Gelen separerades från fröerna och frystorkades innan analyserna utfördes. Med mätningar utförda med *Multi-Angle Light Scattering* bestämdes molekylvikten vara 50–100 kDa. Uppmätt absorbans med spektrofotometer resulterade i en detekterad substans vid våglängd 280 nm. Detta var däremot i en mycket liten koncentration men indikerar på aromatiska aminosyror i protein. Bestämning av lösliga kostfiber utfördes enligt Uppsala-metoden med analys av neutrala sockerrester genom gaskromatografi och uronsyrarester genom spektrofotometri. För att förstå strukturen hos frö-gelen genomfördes ^1H NMR spektroskopi. Gelen bestod upp till 80 % av kostfiber och med ett totalt utbyte på 7 % från uppvägda frön. Resultaten visade att frö-gelen består av en hög andel uronsyra, galaktos och ramnos - ett ramnogalaktouronisk pektin. Pektinet bidrar till en mycket stabil och pålitlig frö-gel som kan användas i stor utsträckning inom livsmedelsindustrin. Möjliga användningsområden är t. ex. kostfiberberikning i livsmedelsprodukter eller vid produktion av glutenfritt bröd vilket testades i denna studie.

Den här uppsatsen visar att frö-gelen från *L. campestre* är en mycket lättextraherad och lättisolerad substans som är luktlös och sannolikt smaklös. Från *L. campestre* är det möjligt att få en substans bestående upp till 80 % av ren pektin, där det enda som krävs är tillgång till vatten och en mixer.

Nyckelord: lösliga kostfiber, pektin, frö-gel, hydrokolloid, fältkrassing, *Lepidium campestre*, oljeväxt

Table of contents

Abbreviations	5
1 Introduction	7
1.1 Aim and objectives	7
1.1.1 Limitations	8
2 Literature review	9
2.1 <i>Lepidium campestre</i> (field cress)	9
2.2 Dietary Fibre (DF)	10
2.2.1 Soluble and insoluble dietary fibre	11
2.3 Gel substances	12
2.3.1 Garden cress	12
2.3.2 Chia seed	13
2.3.3 Guar gum	13
3 Material and Method	14
3.1 Seed samples	14
3.2 Pre-experimental work	14
3.2.1 Gel separation	14
3.2.2 General analysis	15
3.3 Sample preparation	16
3.3.1 Sample preparation with EDTA including dialysis	16
3.3.2 Sample preparation with Ultra-Turrax	17
3.4 Analysis of Dietary Fibre	17
3.4.1 Total dietary fibre	17
3.4.2 Soluble dietary fibre analysis with sample treated with EDTA- and Ultra-Turrax treated samples	18
3.5 Multi-Angle Light Scattering (MALS)	18
3.6 Nuclear Magnetic Resonance (NMR)	18
3.6.1 NMR analysis of EDTA-treated samples	18
3.6.2 NMR analysis of Ultra-Turrax treated samples	19
3.7 Baking with <i>L. campestre</i> seed gum	19
3.7.1 Gluten-free sponge cake	19
3.7.2 Gluten-free yeast leavened bread	20
4 Results	22
4.1 General analysis	22

4.2	Dietary Fibre	23
4.3	MALS	25
4.4	¹ H NMR spectroscopy	26
4.5	Baking with <i>L. campestre</i> seed gum	27
4.6	Gel yield from seed	29
	4.6.1 Samples treated with EDTA	29
	4.6.2 Samples prepared with Ultra-Turrax	29
5	Discussion	30
5.1	Functional properties	32
6	Conclusion	34
	References	35
	Acknowledgement	38
	Appendix 1: ¹H NMR spectrum	39
	Appendix 2: Popular scientific summary	40

Abbreviations

A	Absorbance
Ara	Arabinose
DF	Dietary Fibre
DM	Degree of Methylation
EDTA	Ethylenediaminetetraacetic acid
Fuc	Fucose
G	Gel (sample/substance)
Gal	Galactose
GC	Gas Chromatography
Glc	Glucose
<i>L.</i>	<i>Lepidium</i>
MALS	Multi-Angle Light Scattering
Man	Mannose
NMR	Nuclear Magnetic Resonance
NSP	Non-Starch Polysaccharides
Rha	Rhamnose
rpm	Revolutions Per Minute
SD	Standard deviation
SLU	Swedish University of Agricultural Sciences
TDF	Total Dietary Fibre
UA	Uronic Acid
W	Water (sample/substance)
WHC	Water-Holding Capacity
Xyl	Xylose

1 Introduction

Today *Lepidium campestre* (*L. campestre*) or more commonly called field cress, is a wild species belonging to the *Brassicaceae* family, distributed all over the world. It is an oilseed species with a great potential of becoming a new commercial crop in Sweden for food, bio-diesel and by-products such as animal feed and packaging material. Because of its great potential, *L. campestre* is a part of Mistra Biotech – a program using biotechnology where one of the aims is to domesticate *L. campestre* into a novel oil- and cover crop (Ivarson *et al.*, 2013; Mistra Biotech Annual Report 2014).

As the oil has been extracted from the crop, the residual from the extraction is left in form of a seed cake. This seed cake can possibly be used as livestock feed, but as the seed of *L. campestre* is soaked in water, a clear gel is exuded from the seeds mucilage layer. In this paper, the gel will be referred to as the seed gum or simply as the gel. Other terms used for similar gels are mucilage or mucilaginous gel and hydrocolloids. Similar gels extracted from different seeds are e. g. formed in chia (*Salvia hispanica* L.), garden cress (*L. sativum*), linseed/flax (*Linum usitatissimum* L.) and basil (*Ocimum basilicum* L.). (Andersson *et al.*, 1999; Coorey, Tjoe & Jayasena, 2014; Behrouzian, Razavi & Phillips 2014)

1.1 Aim and objectives

The purpose of this paper is to study the chemical composition of the seed gum extracted from *L. campestre* and to examine if there is a possible field of use for this substance.

1.1.1 Limitations

L. campestre is a potential novel oil crop, earlier studies and research is therefore focusing on the fatty acid content, composition and at the harvest yield and technology of the crop seeds. This paper will limit the study to determination of total dietary fibre and the function and stability of the seed gum. No study will be done on the seed itself. The seed gums function will be tested, but no real rheological study will be executed.

Finally, there will only be one kind of seeds from one harvest analysed and different kinds of varieties within the species *L. campestre* will not be studied.

2 Literature review

2.1 *Lepidium campestre* (field cress)

L. campestre (field cress or also called field pepperweed) belongs to the *Brassicaceae* family and is a wild species that is distributed all over the world (Ivarson *et al.*, 2017). Domestication of the crop is a part of Mistra Biotech: a project founded by the Swedish Foundation for Strategic Environmental Research (Mistra) and the Swedish University of Agricultural Sciences (SLU) - that is focusing on the use of biotechnology for sustainable and competitive agriculture and food systems. The aim for *L. campestre* is to get it to be a combined novel oil- and cover crop. (Geleta *et al.*, 2014, Mistra Biotech Annual Report 2014)

L. campestre is a biennial crop which compared to annual crops, provides higher carbon storage and better soil and water management. With the quality of being a biennial crop, *L. campestre* can be used as a cover crop (catch crop). With the aim of reducing nutrient leaching and soil tillage, the cover crop is sown under cereal crops during the spring. (Mistra Biotech Annual Report 2014)

It has also been shown to have a high seed yield (5-6 tonnes/ha), approximately 30 % higher compared to the average yield of winter rapeseed – that is the oil crop used in Sweden today. Additionally, *L. campestre* is a cold-hardy crop and can therefore be grown in the northern part of Sweden compared to winter rapeseed, that can only be grown in the southern part of the country. (Merker *et al.*, 2010, Ivarson *et al.*, 2013)

Today, oil from the rapeseed family is not sufficient due to its lack of winter hardiness. Domestication of *L. campestre* would be an asset for Swedish oil production - both for human food, livestock feed and bio-diesel consumption (Nilsson *et al.*, 1998; Andersson *et al.*, 1999; Ivarson *et al.*, 2013)

To motivate domestication, it is also important that the whole plant is being used, not only for the main reason - production of oil. After that the oil has been pressed

from the seeds, it is leaving a “seed cake” containing the pulp which is a suitable product to find a new field of use for. Researchers at SLU have taken this in consideration and there have been trials where the seed cake has been used as a component in feed for pigs. The pigs do not seem to notice any difference in the feed taste containing part of the seed cake and it does not seem to have any harmful health effects. (Arefaine, 2016; Mistra Biotech Annual Report 2016)

As it turns out, when in contact with water, the seed and seed cake get slimy and jelly. This is also in line with the study by Andersson *et al.*, (1999), where the aim was to evaluate the chemical composition of the seeds among other crops, where the seeds turned out to be gel forming when soaked in water.

2.2 Dietary Fibre (DF)

Definition and classification of dietary fibre (DF) is an extensively discussed subject. The ground for the classification is based on physiological, chemical and functional properties. DF is an edible part of the plant material, which include non-starch polysaccharides (NSP) lignin and other associated plant substances such as resistant starch. DF is mainly found in the plant cell wall and is resistant to digestion and absorption in the human small intestine, usually with complete or partial fermentation in the large intestine (AACC International, 2001).

The amount and composition of DF found in different food products depend on the food. Foods rich in DF are usually cereals, vegetables, fruits and nuts. (Dhingra *et al.*, 2012)

The content of DF has been shown by Andersson *et al.*, (1999) to be high (414 g kg⁻¹ dry matter) in *L. campestre* seeds when analysed and compared with two wild *Barbarae* species (also belonging to the *Brassicaceae* family). This is also higher than found in rapeseed with a DF content of 180 g kg⁻¹. (Andersson *et al.*, 1999)

The recommended daily intake of DF is 20-35 g/day for a healthy adult. This is a value that the average person does not reach during the day (European Food Safety Authority 2010, Dhingra *et al.*, 2012). DF is important for many reasons, it has shown to have several positive health effects. A high intake of DF has a decreased incidence of several types of diseases by lowering cholesterol, regulate blood pressure and by adding bulk to the diet and stool. (Dhingra *et al.*, 2012)

However, glucosinolates have been found in the seeds of *L. campestre* which has toxigenic and antinutritional impact. The glucosinolate structure found is mainly Sinalbin with a content of 109.9 g kg⁻¹. (Andersson *et al.*, 1999)

2.2.1 Soluble and insoluble dietary fibre

A result caused by the different properties of DF, differentiation of NSP or DF has been done on its solubility in a pH defined buffer. The insoluble NSP is including cellulose, hemicellulose and lignin. While soluble NSP include pectin, gums and mucilage. The solubility decides the fibres' functionality and physiological effects. (Dhingra *et al.*, 2012, Lattimer & Haub, 2010)

The seed gum from *L. campestris* is a soluble dietary fibre. Soluble fibres reduce glycemic response and increase in viscosity, soluble DF has, compared to insoluble, shown to be more beneficial as it provides viscosity and ability to form gels and act as emulsifier. (Mudgil & Barak, 2013)

Pectin

Pectin is a complex of NSP and is a part of the human diet by a normal intake through vegetables and fruit. Fruits that consist of a high amount of pectin in the outer layer of the fruit are e. g. orange, apple, onion, sugar beet and in different citrus species (Mudgil & Barak, 2013, Tamaki *et al.*, 2007). Of the citrus fruit, the pectin is occurring in the peel which contains of about 0.5-3.5 % of pectin (Lattimer & Haub, 2010).

Pectin is also an ingredient in food products as DF-enrichment complement or as an additive in manufactured products for its gelling quality (Dhingra *et al.*, 2012). For this reason, commercially extracted pectin exist and is used when a gelling or a thickening agent is required (Lattimer & Haub, 2010). Pectin has many functional and physiochemical properties proven: e. g. it exhibits textural properties such as viscosity and gelation (Voragen, Beldman & Schols, 2000).

The pectin consists of a complex structure including galacturonans. The structure is composed of a chain, also called the backbone consisting of (1,4)-linked α -D-galacturonic acid residue with different levels of branching that has additional sugars as side chains. The branched part on the backbone is also called "hairy" regions and is substituted with a (1,2)-linked α -rhamnopyranose residue. These regions have shown to affect the gelling behaviour of the pectin, more branching increase the solubility. (Dhingra *et al.*, 2012, Mudgil & Barak, 2013, Voragen, Beldman & Schols, 2000, Renard, Cr  peau & Thibault, 1995, Ridley, O'Neill & Mohnen, 2001)

Arabinose, galactose, mannose and xylose residues are commonly found in the branches of the "hairy" regions of the pectin (Renard, Cr  peau & Thibault, 1995). The behaviour of the gelation in pectin is also influence by the degree of methylation (DM), which will influence the field of use for a specific pectin. DM will affect the ability of the pectin to form gels with high amounts of sugar, acid or with the presence of calcium. It is therefore important to know the DM of the specific pectin

when used in the food industry. A part of the galacturonic backbone is carbonyl groups (COO⁻), where some of the carbonyl groups are methoxylated. DM is depending on the variation of the content of methoxylation and carboxyl group of the pectin. (Voragen, Beldman & Schols, 2000, Tamaki *et al.*, 2007, Sharma *et al.*, 2015, Rosenbohm *et al.*, 2003)

It is shown that pectin itself stands for a part of the health claims for DF. Pectin is shown to bind the cholesterol and bile acid in the gut and as a response to that, promoting their excretion, which results in lower blood cholesterol. The health effect is at least partly due to the pectins gel-forming capacity. (Mudgil & Barak, 2013)

2.3 Gel substances

Hydrocolloids are a mixture of viscous polysaccharides that can include derived exudates from plants, seed gums and seaweeds extract (Mudgil & Barak, 2013). The hydrocolloids are widely used in the food industry as enrichment or as ingredient with qualities that improve performance characteristics in food production and food development (Coorey, Tjoe & Jayasena, 2014). Below are three selected hydrocolloids that all can be compared to *L. campestre* seed gum. The selections are based on relationship to *L. campestre* but also depending on the use and how common the substance is today.

2.3.1 Garden cress

L. sativum or garden cress is also a member of the *Brassicaceae* family and closely related to *L. campestre*. *L. sativum* possess the same quality of having seeds that being gel forming when in contact with water. *L. sativum* is however, a domesticated crop and widely used in food consumption, and therefor also more studied than *L. campestre*. (Gokavi, Malleshi & Guo, 2004, Behrouzian, Razavi & Phillips, 2014)

L. sativum is a rapidly growing plant that requires minimal agricultural resources. According to Diwakar *et al.* (2008) it can be harvested within 70–90 days and give a calculated seed yield up to 800–1000 kg/ha. The seed gum from *L. sativum* has been shown to have high molecular weight (540 kDa), be tasteless and consist of many different sugar residues such as; 38.9 % mannose (Man), 19.4 % arabinose (Ara) and 8 % galactouronic acid, with a total dietary fibre (TDF) content up to 77 %. (Karazhiyan *et al.*, 2009)

2.3.2 Chia seed

Chia (*Salvia hispanica*. L.) seeds have also a clear gel surrounding the seeds when soaked in water. Despite this common quality with *L. campestre* and *L. sativum*, Chia instead belongs to the *Lamiaceae* family (Ramos *et al.*, 2016). The seed gum from chia seeds has shown to have a high molecular weight and of its polysaccharides, mainly consist of xylose, glucose and uronic acid (Lin, Daniel & Whistler, 1994).

The Chia seeds have been shown to be rich in both DF (34.6 %), protein (24.6 %) and oil (32.2 %). The seeds are conventional used product because of its health claims and gel building seeds, however, research concerning chia seed gum and its functional properties is very few. (Coorey, Tjoe & Jayasena, 2014, Alfredo *et al.*, 2009)

2.3.3 Guar gum

Guar gum is extracted from the seeds of guar- or cluster bean (*Cyamopsis tetragonoloba*). It is widely used by the food industry in form of guar gum flour or powder as thickener, emulsifier and stabilizer in different food products. Other fields that the guar gum is occurring are e. g. pharmaceuticals, paper, textile, explosives and cosmetics industry. The properties of guar gum as a gel-forming substance are due to a high number of high molecular weight polysaccharides in form of galactomannan (mannose and galactose residues). The amount of total DF (TDF) in dry weight of guar gum is 52-58 % where 26-32 % of this amount representing soluble DF. (Joshi, Bhokre & Rodge, 2015; Mudgil, Barak & Khatkar, 2014)

3 Material and Method

3.1 Seed samples

Seeds from *L. campestre* were provided from the SLU in Alnarp, 2015 and stored vacuumed packed in room temperature until used in this study. The seeds were sieved and separated from unwanted plant material from the crop by using a vibratory sieve shaker, Retsch AS200, with mesh size of 1 and 2 mm and a 600 μm mesh that intercepted the seeds.

3.2 Pre-experimental work

Water and seeds from *L. campestre* were added together in a beaker and mixed by hand with a spoon to form the gel that surrounds the seeds. It was then left in refrigerator overnight.

3.2.1 Gel separation

The water surrounding the gel and seeds were more viscous than from start as water alone and was therefore separately filtered and included as a second sample. This is referred to as “W” (water sample) in Figure 1.

Separation through freeze drying

The seeds, including the surrounding gel was referred to as one sample and the second sample the viscous water surrounding the gel. These two samples were frozen and freeze-dried in Edwards Freeze Dryer Modulyo.

Separation through mixing

The steps of separation through mixing of the gel is demonstrated in Figure 1. The viscous water sample (W) was first separated from the seeds and gel by filtration (step 1). When separate the stable gel surrounding the seeds - without destroying the seeds, the sample was separated with DuPont Instruments Sorvall Omni-Mixer. The sample was repeatedly mixed a couple of times in low power setting and then filtered through a glass funnel with open small holes (step 2-3).

In attempt to make both solutions more transparent, centrifugation was executed in 600 mL plastic containers at 3000 rpm in 15 min. The centrifugation did not result in any supernatant or pellet. Instead, the viscous water sample was clarified with filtration through a glass filter funnel number 2 with vacuum. This was not possible with the more viscous gel sample. To get a dry sample to analyse, both solutions were freeze-dried in Edwards Freeze Dryer Modulyo.

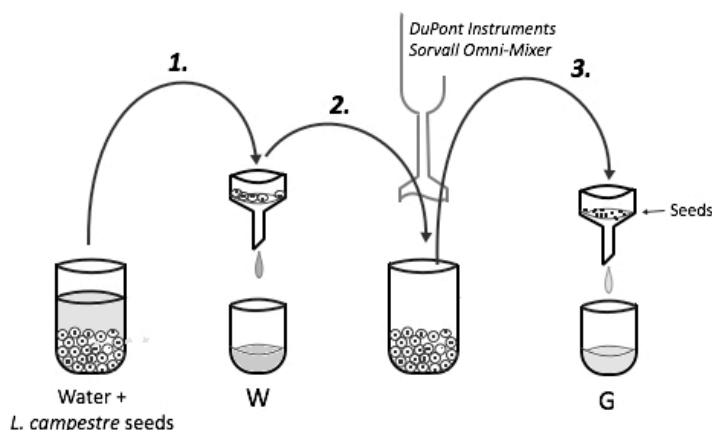


Figure 1. Demonstration of the separation of the two samples: W for water substance and G for gel substance. In step one the seed and the stable gel bubble surrounding the seeds stays in the funnel while the viscous water is filtered through. After mixing the seed and gel with mixer, the gel is being filtered through the glass filter funnel, leaving the seeds in the funnel.

3.2.2 General analysis

By using Metrohm 827 pH lab the pH was at a gel and a water solution that been separated through mixing but without the freeze-dried treatment. With freeze-dried samples from both water and gel solution (from samples prepared with both EDTA and Ultra-Turrax, see 3.3 *Sample preparation*), light absorbance spectrum was measured with UV-1800 Shimadzu for the interval of 200 – 800 nm. Sample (10

mg) was dissolved in 3 mL H₂O and put in a 70 °C water bath for better dissolving. The water samples were in the water bath for just a few minutes while the gel samples were left for 20 min.

To test if the two solutions were affected by high temperature, 3 mL of each sample was added in separate glass tubes and put down in a boiling water bath for 5 min and observed. Precipitation through contact with alcohol was also tested by adding 3 mL of 99.5% EtOH stepwise five times in a 3 mL of each water and gel sample.

A solution of 0.02 M CH₃COOH and 0.02 M NaOH was made to investigate if the seeds were affected by a pH dependent solution instead of water. Each 0.02 M solution (40 mL) was added to 0.5 g untreated seeds in two glass tubes and the samples were properly shaken and observed.

3.3 Sample preparation

Two different kinds of sample preparations were performed to make the gel less viscous, with and without Ethylenediaminetetraacetic acid (EDTA) treatment as a chelating agent.

3.3.1 Sample preparation with EDTA including dialysis

Together with 250 mL H₂O, 10.00 g seeds were mixed with a spoon and then left in the refrigerator overnight. The seed and water was filtered with a glass funnel in to a 1000 mL E-flask and washed together with 150 mL water. Remaining seed with gel was mixed in DuPont Instruments Sorvall Omni-Mixer in low power setting with additionally 150 mL H₂O stepwise. The separated gel solution was filtered through the same glass funnel and transferred in to a separate 1000 mL e-flask. EDTA was added in both samples to a concentration of 0.05 M and left for 3 h with repeated mixing of the sample by swirling around the E-flask.

Dialysis was performed in Spectrum - molecularporous, membrane tubes overnight for approximately 20 h with running deionised water. In each sample, 0.5 mL CHCl₃ was added directly in the tube to avoid microbial growth. The water sample was filtered with vacuum through a glass filter number 2. The gel sample was too viscous to be filtered through a glass filter number 1 with larger pores and therefore left turbid. All samples were stored in a freezer for 24 h. Frozen samples were then put in the Edwards Freeze Dryer Modulyo for a couple of days until totally freeze-dried.

3.3.2 Sample preparation with Ultra-Turrax

The seeds were sorted out by hand from small unwanted residues (that could not be sorted out by the sieve shaker) and 10.0083 g seeds were blended with 250 mL H₂O and left in refrigerator for 1 h. The gel and viscous water solution were separated. The viscous water sample was separated by filtration through glass funnel together with 100 mL H₂O. The seeds with gel attached were mixed in turns with additional 100 mL H₂O in the DuPont Instruments Sorvall Omni-Mixer and the gel separated from the gel-free seeds with filtration through the glass funnel.

Both samples were mixed in Ultra-Turrax T25 in 2 min each at 13 500 rpm. After mixing, the samples were put in a boiling water bath for approximately 20 min – until the samples got a temperature of 90 °C. Last, the samples were stored in freezer overnight, before put in the Edwards Freeze Dryer Modulyo.

3.4 Analysis of Dietary Fibre

3.4.1 Total dietary fibre

Duplicate samples were taken for freeze-dried samples from the pre-experimental gel separation preparations without mixing. This included the seeds in one of the samples, the seeds were included in the performance until after the hydrolysis. Execution of the soluble analysis was performed according to The Uppsala Method, AOAC International with minor rectifications (Theander *et al.*, 1995). The rectification that was done during hydrolysis were that the 3 mL of 12 M H₂SO₄ was diluted in 74 mL H₂O before mixed with the dry sample and standard.

Soluble dietary fibre

Two samples from the freeze-dried material did not undergo the whole treatment of total dietary fibre analysis preparation in the first trial. These two were instead left out from the removal of starch and first included during hydrolysis, the hydrolysis was performed according to the method for soluble dietary fibre.

All six samples, both soluble and insoluble were analysed by gas chromatography (GC) for neutral sugar residues. Determination by colorimetry was done for uronic acid (UA).

3.4.2 Soluble dietary fibre analysis with sample treated with EDTA- and Ultra-Turrax treated samples

The analysis was performed at the freeze-dried material from the gel preparation with EDTA and with freeze-dried material treated with Ultra-Turrax. From each EDTA sample, 3-10 mg, and 10 mg from Ultra-Turrax sample, was weight with two duplicates (“1” and “2”) and hydrolysed as soluble fibres according to The Uppsala Method. Analysis for neutral sugar residues were done in GC and determination of UA by spectrophotometry (Theander *et al.* 1995).

3.5 Multi-Angle Light Scattering (MALS)

Measurement with Multi-Angle Light Scattering (MALS) was performed on freeze-dried samples treated with EDTA. From gel sample, 0.0107 g was dissolved with 2 mL 0.1 M NaNO₃ including 0.02 % NaN₃ and mixed with Kinematica AG Polytron-PT3000. First it was mixed 1 min at 15 000 rpm and then additionally 1 min in 19 000 rpm. A supernatant was separated through centrifugation of the sample in Eppendorf tubes with centrifuge adjustment at 10 000 rpm in 15 min.

For the water sample material, 5.7 mg sample was dissolved in 1 mL 0.1 M NaNO₃ including 0.02 % NaN₃. No centrifugation was needed. Both samples were filtered through 0.45 µm down into vials and analysed with high-performance size-exclusion chromatography columns (OHpak SB-806M HQ, OHpak SB-804 HQ, and OHpak SB-803 HQ, Shodex, Showa Denko KK, Miniato, Japan) kept at 35 °C. Detectors were refractive index and multiple-angle laser light scattering (Dawn DSP, Wyatt Technology Corp., Santa Barbara, CA).

3.6 Nuclear Magnetic Resonance (NMR)

3.6.1 NMR analysis of EDTA-treated samples

NMR spectroscopy was performed on the freeze-dried samples prepared and treated with EDTA. 0.0064 g of the water sample was dissolved in 1 mL H₂O. For the gel sample, 0.0072 g dry matter was dissolved in 2 mL H₂O and run through Kinematica AG Polytron-PT3000 at 18 000 rpm in 3 min.

Both water dissolved samples were centrifuged 15 min at 10 000 rpm. The gel sample was centrifuged two times before the supernatant could be separated with help from a Pasteur pipette. Supernatant from both samples including the pellet from the gel sample was put in Edward Freeze Dryer Modulyo for a second freeze drying. Before analysed in NMR, the three samples were dissolved in 700 µL D₂O each. ¹H

spectra were recorded at 80 °C using 90° pulse angle, and chemical shifts determined with the water signal as reference at 4.21 ppm.

3.6.2 NMR analysis of Ultra-Turrax treated samples

Dry matter sample from sample prepared with Ultra-Turrax was taken direct from the freeze drier and weight in Eppendorf tubes. From gel sample, 7.7 mg was taken and from water sample 7.1 mg. Both samples were mixed with 700 µL D₂O, strongly vortexed with high speed in Vortex-genie 2 and centrifuged at 14 000 rpm in 20 min. Another 700 µL of D₂O was added in the gel sample and the performance of vortex and centrifugation was repeated with only the gel sample to get a clear difference between supernatant and pellet. ¹H spectra were recorded at 80 °C using 90° pulse angle, and chemical shifts determined with the water signal as reference at 4.21 ppm

3.7 Baking with *L. campestre* seed gum

A possible use for the seed gum of *L. campestre* could be as a thickener in gluten free product. This was tested for both bread baked with yeast and sponge cake recipe baked with baking soda.

3.7.1 Gluten-free sponge cake

The used seed gum in the baking process of sponge cake was made of 6.7 g seeds, mixed together with 100 g H₂O. After mixing with DuPont Instruments Sorvall Omni-Mixer of both the water and gel part, it was filtered through a glass funnel and 35 mL gum was used in the mixture instead of using milk that is commonly used in regular sponge cake. The recipe is shown in Table 1.

Table 1 *Sponge cake recipe baked with seed gum from the seeds of L. campestre.*

Ingredients	Recipe
Sugar	100 g
Eggs	1 egg
Butter	33.1 g
Seed gum	35 ml
Potato flour	25.1 g
Buckwheat	46.6 g
Almond powder	16.6 g
Baking soda	3.3 g

The egg, sugar, melted butter and gum was mixed together by an electric kitchen beater before the dry ingredients were added. The cake was baked in 175°C for 40 min in a baking pan with almond powder. When the cake was done it was cooled up-side down before it was separated from the baking pan.

3.7.2 Gluten-free yeast leavened bread

Baking with buckwheat

Three different breads were baked, one with the seed gum from *L. campestre*, a second recipe with the seed gum including the seeds and a third recipe without seed or seed gum - just water. The used seed gum was produced according to the same procedure as for the sponge cake. The exact recipe is shown in Table 2.

The seed gum, seed gum including seeds or the water was first blended with yeast and honey before the dry material was added in the dough. Each dough was covered with plastic film and towel and left to rest for 1 h and 45 min. After resting, each dough was gently stirred and moved over to separate baking pans that had been floured with corn flour. A final rest and fermentation was performed under towel for 1 h before baking of the bread in 150 °C for 1 h in the middle of the oven.

Table 2 *Three different recipes from the first baking test of gluten free bread. Recipe 1 is with only seed gum, Recipe 2 with water and seeds and Recipe 3 with water. All amounts are given in gram*

Ingredients (g)	Recipe 1	Recipe 2	Recipe 3
<i>L. campestre</i>	-	9.5	-
Water	-	143	143
Seed gum	143*	-	-
Yeast	4.8	4.8	4.8
Honey	5.0	4.9	5.1
Corn flour	42.0	42.0	42.0
Buckwheat	50.0	50.0	50.0
Salt	1.5	1.5	1.5

* 9.5 g seeds from *L. campestre* was stirred with a spoon in 160 g water and mixed in DuPont Instruments Sorvall Omni-Mixer and filtered together with additionally 23 g water for washing. The filtrated seed gum was weight separately and used in the baking.

Baking with corn flour

A second baking test was performed without buckwheat and instead just corn flour - to test the properties of the seed gum from *L. campestre*. For the dough to better suit the volume of the baking pan used, the recipe was adjusted to 75 % of the amount from the first baking test. In the baking test with only corn flour, two different recipes were done. One with the seed gum and one control without seed gum and instead only water (see recipe 1 and 2 in Table 3).

The water or the seed gum was blended with yeast and honey before the corn flour and salt was added. The doughs were then left for 1.5 h to ferment under plastic film and towel. Each dough was gently stirred and moved to a baking pan floured with corn flour and then left under towel for 1 h. The baking of both breads was done in the middle of the oven in 175 °C for approximately 1 h.

Table 3 Recipe 1 is included seed gum and show the weight in gram of every ingredient added and recipe 2 the weight of ingredient used in the dough without *L. campestre*

Ingredients (g)	Recipe 1	Recipe 2
Water	-	107.1
Seed gum	107.1*	-
Yeast	3.6	3.6
Honey	3.6	3.6
Corn flour	70.0	70.0
Salt	1.0	1.0

* 7.1 g seeds from *L. campestre* was stirred with a spoon in 100 g water and mixed in DuPont Instruments Sorvall Omni-Mixer and filtered together with additionally 20 g water for washing. The filtrated seed gum was weight separately and used in the baking.

4 Results

4.1 General analysis

The measured pH of the gel phase was 5.75 and the water phase 5.36. None of the samples water or gel phase were affected by a pH depended factor such as CH_3COOH and NaOH , both samples continued to be viscous. The seed gum in the NaOH did however change in colour from colourless to orange-red that increased its colour intensity with time.

The samples viscosity was not affected by heat but in the gel sample, small air bubbles were developed. They did however, not affect the samples viscosity and were probably due to air in the solution and not caused by the gel. No notable viscosity change was detected when the seed gum with or without the seed was stored in refrigerator over a longer time. The seed gum was not thawed after stored in the freezer, therefore no statement can be done for the gel properties after thawing. The gel did, however, re-emerge as the dry matter was soaked in water again, after freeze-drying treatment.

Addition of 99.5 % EtOH did not have any effect on the samples viscosity, the highest concentration of EtOH reached after 3 mL stepwise adding, was 89 % (total 15 mL added EtOH). The samples were left over night and no change could be seen after 24 h either.

As the freeze-dried sample was soaked in water it was not a clear solution and not optimized for measuring in spectrophotometry - therefore the base line is hard to define and at different levels for the different samples in the UV spectra. The measured absorbance in light absorbance spectrum resulted in a detected substance at a wavelength of approximately 280 nm for all measured samples. This is presented in the light absorbance spectra in Figure 2.

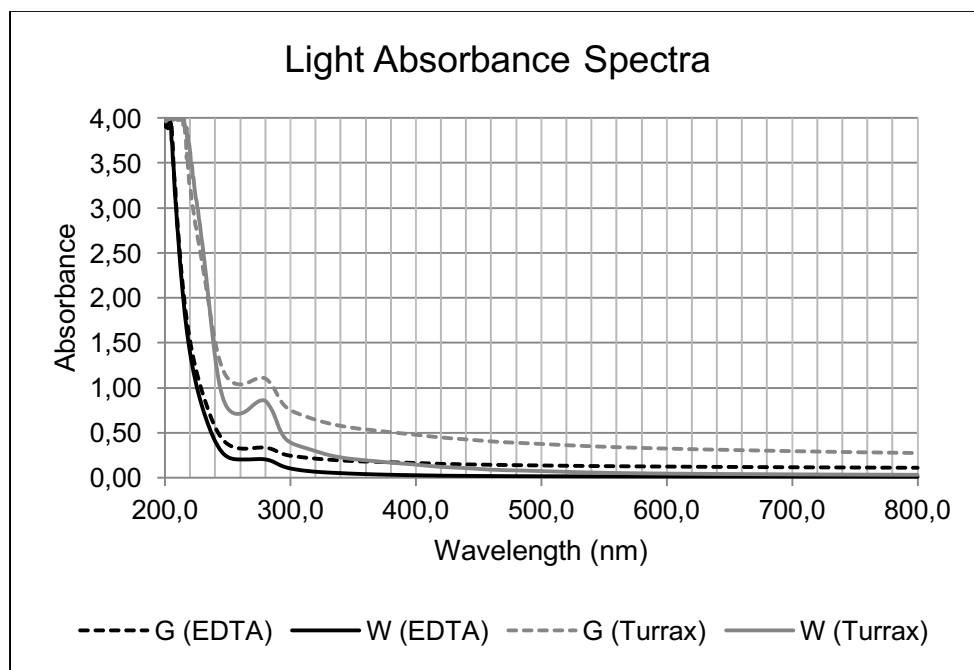


Figure 2 UV spectra of gel (G) and water (W) samples from freeze-dried material prepared with both EDTA (EDTA) and samples treated with Ultra-Turrax (Turrax). All samples show a small peak at approximately 280 nm.

Peaks at 280 nm are typically indicating aromatic amino acids in protein. A brief calculation of the concentration using the formula $(A_{280} / \epsilon_{\text{percent}}) \times 10 = c_{\text{mg/mL}}$, give the concentrations presented in Table 4 for each sample analysed in spectrophotometry. (Thermo Scientific, 2013)

Table 4 Calculated concentration of samples analysed in spectrophotometry at absorbance analysed at wavelength 280nm

Sample	Absorbance ₂₈₀	Concentration (mg/mL)
G _{EDTA}	0.335	0.508
W _{EDTA}	0.201	0.304
G _{Turrax}	1.100	1.666
W _{Turrax}	0.853	1.292

4.2 Dietary Fibre

In Table 5 the result from the first experimental determination is presented. Incorrect documentation of the weight of sample W* is giving a calculated value of total DF (TDF) at 156.2 %. In this analysis, the seeds were included in the dry matter weight for the gel samples (G*, G₁ and G₂) during the determination of TDF. Therefore,

Klason lignin, that is determined gravimetrically, is not included in the determination. G* (and W*) is the sample that did not undergo the preparations of removal of sugars and starch before hydrolysis and G₁ and G₂ are duplicates (as well as W₁ and W₂).

Table 5 *Content and composition of total sugar residues from first analysis with samples from pre-experimental fractions. The values of the neutral sugar and uronic acid residues is the relative percentage (% of TDF), and the value for TDF is the absolute percentage of the dry matter sample*

Sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	TDF
W*	18.1	-	-	9.9	-	40.9	13.9	17.2	156.2
G*	8.3	-	20.5	8.2	-	15.3	28.0	19.8	73.3
W ₁	21.0	-	-	8.5	21.4	40.7	-	8.4	50.3
W ₂	19.4	-	-	8.3	24.7	39.5	-	8.1	50.1
G ₁	7.9	-	24.5	6.7	21.6	20.4	8.6	10.4	11.8
G ₂	8.2	-	23.7	7.1	22.5	19.2	9.0	10.3	12.2

*Water (W) and Gel (G) sample performed without the preparation with removal of sugars and starch.

The biggest difference in neutral sugar residues between water- (W) and gel (G) samples is the absence of Arabinose (Ara) and Glucose (Glc) in W- samples. This is also corresponding to other determinations performed with and without EDTA, where Ara and Glc is absent or present in very small amount in W, compared to G-samples (Table 6 and 7).

Table 6 *Content and composition of total sugar residues from first analysis with EDTA sample. The values of the neutral sugar and uronic acid residues is the relative percentage (% of TDF), TDF is the absolute percentage of the dry matter sample*

Sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	TDF
<i>3 g EDTA sample</i>									
W ₁	12.1	-	1.7	5.5	-	26.0	-	54.6	26.7
W ₂	11.1	-	1.9	5.3	-	23.6	-	57.9	19.2
G ₁	9.5	-	10.0	5.6	-	23.7	3.7	47.5	20.3
G ₂	7.3	5.3	9.0	4.6	-	23.0	3.7	47.2	20.5
<i>10 g EDTA sample</i>									
W ₁	10.9	-	1.9	5.4	0.6	25.6	1.1	54.4	18.3
W ₂	10.5	-	1.8	5.0	0.5	25.5	0.7	56.0	34.2
G ₁	8.4	-	11.0	5.4	1.0	24.2	2.9	47.1	20.1
G ₂	8.5	-	10.5	4.9	1.3	23.8	2.6	48.4	17.4
<i>Average</i>									
W	-	-	-	-	-	-	-	-	24.6 ± 6.4
G	-	-	-	-	-	-	-	-	19.6 ± 1.3

Determination performed at the EDTA treated samples are presented in Table 6. A calculated average and standard deviation (SD) of the TDF amount in W-sample is 24.6 ± 6.4 and 19.6 ± 1.3 in G-sample. The average is not calculated for the neutral sugar residues because the values of the two determinations is the relative percentage of each determination, meaning that the values depend on each other and the absence of e. g. Mannose (Man) in determination with 3 g sample will increase the other relative values.

All three determinations (EDTA and Ultra-Turrax sample) show a high value of UA with consistent proportions: 55.6 ± 1.3 for W- and 47.8 ± 0.8 for G-samples. In Table 7 the result from the Ultra-Turrax treated samples without EDTA is shown. This determination shows a much higher amount of TDF than for the EDTA-treated samples. The average and SD for TDF is 78.6 ± 2.8 for W- and 61.5 ± 4.4 for G-samples.

Table 7 Content and composition of total sugar residues from analysis of Ultra-Turrax treated sample. The values of the neutral sugar and uronic acid residues is the relative percentage (% of TDF), and the value for TDF is the absolute percentage of the dry matter sample

Sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	TDF
W ₁	10.9	-	2.3	5.3	0.5	25.9	0.9	54.2	81.4
W ₂	9.8	-	2.4	5.1	0.5	25.3	0.8	56.3	75.7
G ₁	7.7	-	11.1	4.8	1.2	24.9	3.1	47.2	57.2
G ₂	8.4	-	8.2	5.2	1.2	25.2	2.6	49.2	65.9
<i>Average</i>									
W	10.3	-	2.3	5.2	0.5	25.6	0.8	55.3	78.6 ± 2.8
G	8.1	-	9.7	5.0	1.2	25.0	2.9	48.2	61.5 ± 4.4

Consistent for all analysis regarding W is that it has high proportion of UA, galactose (Gal) and rhamnose (Rha). G on the other hand is consistently high in the proportions of UA, Gal, Ara and Rha.

4.3 MALS

The result from MALS-analysis is presented in Figure 3. The gel is presented as the dashed lines and the water sample in regular lines. The grey lines for both gel and water sample represent the signal from the light scattering while the black line is the concentration (mg/mL).

Peaks of the grey lines that are eluted at 16 mL are from light scattering of polymer aggregates in the samples, which is not detected by the refractive index detector measuring the concentration.

The molecular weight is marked with small grey rings in the figure. This analysis shows that the samples have the same size and molecular weight; from 50 to 100 kDa due to the same retention volume of the two samples.

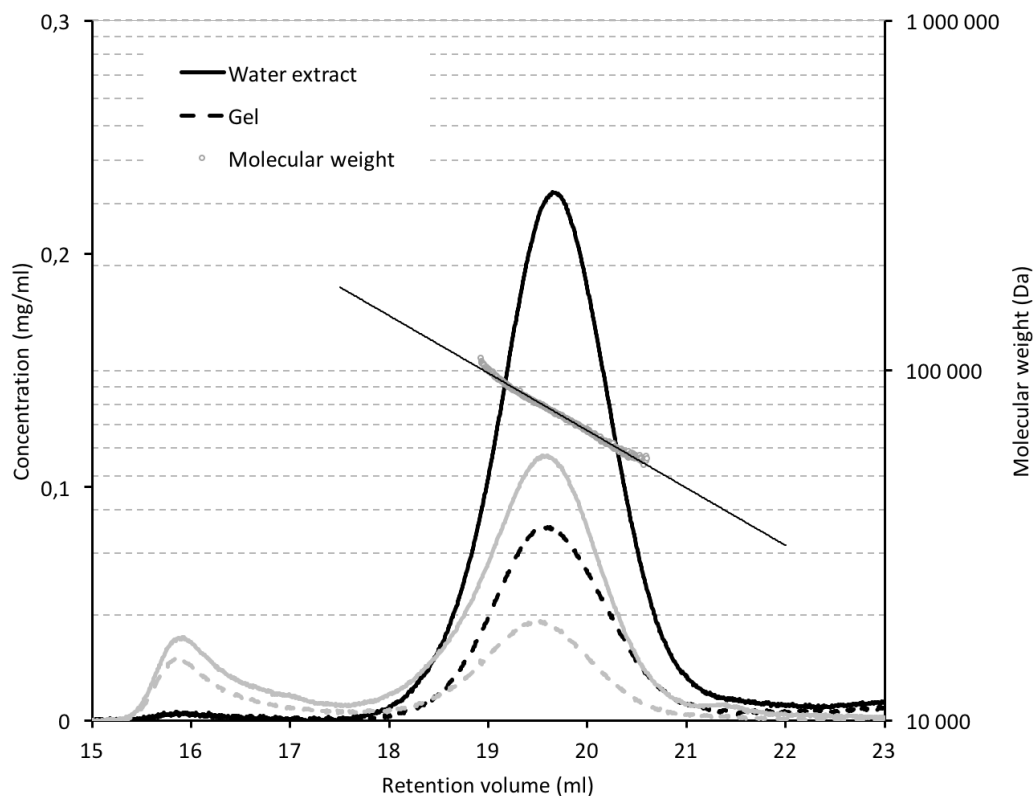


Figure 3 Results from MALS-analysis. The figure is including both gel (dashed lines) and water sample (regular lines). The grey lines represent the signals from light scattering and the black lines represent the concentration (mg/mL).

4.4 ^1H NMR spectroscopy

The first analysis with NMR was performed on the EDTA-treated samples. This resulted in a NMR spectrum that shows big and broad peaks at 3-5 ppm, making it difficult to determine anything in the sample. The NMR-spectra is attached in Appendix 1, (n. b that the scale is not set in relationship to the water signal and should therefore be adjusted approximately one ppm to the right).

The second NMR analysis with Ultra-Turrax-treated sample resulted in a much cleaner and clearer spectra. Figure 4 is presenting the part of the NMR spectra for

the W-sample, where the signals from the anomeric protons appear. Theoretically, the anomeric protons give doublet signals, but residues with α -configuration have such a small coupling constant that they appear as singlets in a polysaccharide with broad signals. UA in pectin is one example of that. With this said, UA that is representing the highest percentage of TDF seems to be represented in two signals, first at 5.0 ppm with an integral of 1.0257 and a second signal at 4.6 ppm with an integral of 1.6250. Rha that also has α -configuration is probably the 1.000 big signal at 5.3 ppm. Finally, there are two signals with β -configuration at 4.4-4.5 ppm which, regarding to the TDF determination is Gal, that have β -configuration.

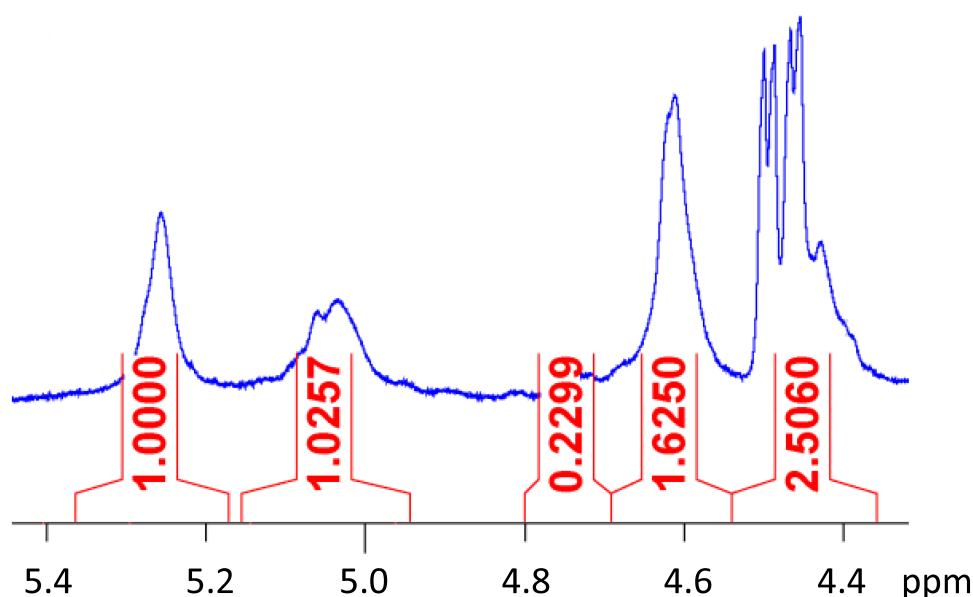


Figure 4 NMR-spectrum from analysis of sample prepared without EDTA in the sample.

4.5 Baking with *L. campestre* seed gum

L. campestre did not have any visible effect that could negatively influence the work of the yeast or the baking powder. Likewise, it did not seem that the seed gum was affected in any way by the added sugar (from the honey) or salt.

No sensory evaluation of the bread or sponge cake was done, only observation and comparison between the different breads. All the bread smelled the same and had the same colour. During first trial with three different recipes (Table 2) it seemed like the buckwheat in fact had a too good baking capacity by itself. Therefore no significant, visible difference of the texture or volume between the breads could be observed.

When baking with 100 % corn flour, difference between the seed gum and the water containing dough (recipes in Table 3) could be observed already at the mixing of ingredients. As it can be shown in the photography in Figure 5, the dough containing the seed gum of *L. campestre* shows a much better water-holding capacity (WHC) than the dough only containing water. In the dough without seed gum, the water is being separated from the rest of the ingredients and is located as a layer in the bottom.



Figure 5 The photograph is showing the dough from baking with corn flour in the start of leavening. To the right is the dough containing seed gum from *L. campestre* and to the left, the dough only containing water (Recepie 1 and 2 from Table 3).

This can also be observed after baking in oven, as shown in the photograph of a cross-section cut of the two breads in Figure 6. The bread to the left in the figure is containing seed gum. This bread shows of an even moist texture all-over the bread, indicating of a good WHC also during the high temperature at 175 °C. To the right is the bread without seed gum which does not possess the same quality of WHC. In the upper part, the bread is dry and compact and in the bottom of the bread wet and moisty. The bread was also very hard to get out of the baking pan due to the moist, which was no problem for the seed gum containing bread.



Figure 6 Photograph of a cross-section cut of the baked bread with corn colour. To the left is the seed gum containing bread and to the right the bread baked with water without seed gum.

4.6 Gel yield from seed

Weight of the freeze-dried sample of both the gel and water sample was taken and the yield of gel from the *L. campestre* seeds was calculated.

4.6.1 Samples treated with EDTA

Amount of seeds from the beginning was 10.00 g and the weight of freeze-dried gel sample was 1.5508 g and water sample 0.5245 g. This gives a final gel yield of 20.8 %, but also includes residual content of EDTA

4.6.2 Samples prepared with Ultra-Turrax

Amount of seeds from beginning was 10.0083 g blended with 450 mL H₂O that resulted in 0.5029 g freeze-dried gel sample and 0.2340 g from the water sample. This gives a yield of 7.4 % extracted from the seeds of *L. campestre*.

5 Discussion

For the obvious reason that EDTA is left in the sample after dialysis, the calculated yield of that batch shows an incorrect and unreliable result at 20.8 % gel yield. Instead is the yield calculation from the Ultra-Turrax treated sample more reliable. In this case, the yield is measured to 7.4 % dry matter gel substance from measured *L. campestre* seeds. This is e.g. a higher yield than reported by Wennerberg *et al.* (1991) for Linseeds, with a yield at 4 %. It is also higher than what is reported for citrus species by Tamaki *et al.* (2007) with a yield at 4.1 % from the pectin rich peel of *Citrus depressa* and recorded 3-4 % yields for lemon.

Different experimental trials of extracting the seed gum from the seeds were carried out to get the seed gum in a clear and more easily manageable form. Several different determinations of neutral sugar residues and UA residues was performed on the fractions obtained (Result in Table 5-7). In Table 5 the result from the first experimental determination is presented. A wrong documentation and weighing of the W* sample has occurred, probably caused by the objective to weighing a very small amount of sample. The freeze-dried samples were very light and electrostatic and therefore, hard to weigh correctly. This can lead to misleading and incorrect results of the individual residues as well for the TDF amount. The relative composition on the other hand seems to be more consistent and reliable.

The amount of TDF, shift significantly between EDTA and Ultra-Turrax treated samples. This is probably due to an incomplete dialysis of the samples including the chelating agent EDTA where some EDTA still is in the samples, making the proportion of TDF lower in the determination. This also corresponds to the NMR-spectrum for the two NMR-analyses. First analysis with EDTA samples, gave several big peaks that overshadow the NMR-spectrum (Appendix 1). The high amount of EDTA in the sample made it hard to study the signals that was made from the actual component from the seed gum. EDTA samples was also the hardest to weigh correctly due to their electrostatic character which is probably due to a bigger volume of the frozen sample before freeze-drying. Some part of the bigger volume can be

caused by the higher amount of water added together with the seeds during preparation. However, the increased volume is most likely occurring of account of osmosis, where water is leaking in to the tube during dialysis.

With this said, the more reliable result is the latter analysis for DF, where a larger amount, without seeds and without EDTA is used. Both the seed gum and the water surrounding the seed gum consist of a high amount of soluble DF.

What is proven in this study is also the importance of the water surrounding the gel, not only the gel-bubble itself. As shown in the determination of TDF, the water has a higher TDF amount than the gel-sample. Making also the water an important substance for further uses. The water sample do also have a higher relatively level of Gal and UA. The one thing that the water substance does not have is Ara residues which do not seem to leave the gel. Thus, this seed gum appears to contain two different pectic substances.

The NMR is consistent with the determination of TDF, where it is possible to distinguish the signals due to the configuration of the residues. NMR together with the determination of TDF confirms that the substance is a rhamnogalacturonic pectin containing branched regions of Gal, but also with branched regions of Ara in the gel-solution.

Even though the Ultra-Turrax treated sample is proven to be more reliable. In MALS-analysis, however, only the EDTA-treated sample was analysed. When mixing the sample in Ultra-Turrax the molecules in the sample is cleaved and the sample will therefore not give a fair result in MALS, when determine the molecular size. In MALS-analysis (Figure 3), the most important and clear result is that the polymer in the sample aggregates which results in a signal from light scattering at 16 mg. This indicate a will in the gel to stay in aggregate form in the sample, which is a god quality of a hydrocolloid substance for food applications. This aggregation factor is also a well-known factor for other studied pectic substances (Taylor & Francis Group, LLC, 2006). The molecular weight shows that the water and gel sample has the same molecular weight because of the same interval of the peaks in retention volume. However, during preparation of the sample this is likely to have been affected. The force that is needed to make the sample acceptable for MALS-analysis is probably destroying the big molecules in the sample, making the result less reliable. The molecular weight is therefore, probably higher than the measured value of 50 to 100 kDa.

The functional properties of a pectin are dependent on its molecular weight. It has also been shown that pectins with the same molecular weight can exhibit differences in e. g. neutral sugar content and branching, which can increase the complexity of the solution even more.

5.1 Functional properties

The properties of the seed gum were overall consistent during the general analysis: heat treatment as well as cold treatment and with the influence of pH and alcohol, no notable change in the seed gums viscosity or behaviour could be observed. This indicate a stable gum. During the baking with the seed gum, the gum was also exposed to both salt and sugar and did not change in appearance there either. One important observation was also that the seed gum itself, did not have any negative effect on the yeast or the baking powder.

The results from light absorbance spectra show consistent peaks at wavelength 280 nm for all samples (Figure 2). Both EDTA and Ultra-Turrax treated samples show similar spectrum, this indicates that there is no EDTA that gives any misleading peaks or results in the light absorbance spectrum. Both samples that was not treated with EDTA gives however a more diffuse result because of an unclear sample and baseline.

Peaks at 280 nm are typically indicating aromatic amino acids in protein. This could also be an indication of glucosinolates that Andersson *et al.* (1999) found in the seeds of *L. campestre*. Glucosinolates consist in many different structures, Andersson *et al.* (1999) found mainly the glucosinolate Sinalbin in *L. campestre*. Sinalbin is however, shown by Müller *et al.* (2001) to be detected in UV spectrum at a wavelength of 224 nm. With this knowledge, you could probably exclude the possibility that the seed gum of *L. campestre* consist of the toxigenic and antinutritional impact of glucosinolates. Which is a positive quality of the seed gum if it is going to be used in production of human food. If the seed is going to be used in food production however, this is something that needs to be reduced or eliminated from the crop before further practice.

In further research of the seed gum from *L. campestre* it would not only be interesting but also very important to know more about the rheological properties of the gum. A more defined method for testing the rheology (texture and viscosity) to be able to compare it with other similar hydrocolloids that is used in the industry today. Further, analysis of the seed cake would be interesting - not only the pure seeds that have been used in this study. It would also be interesting to study different varieties within the species *L. campestre*, to see if there are any differences within the species regarding seed gum yield, amount of TDF, neutral sugar residues and the pectin composition.

Another part that also would be interesting to study if the seed gum will be used in future food production, is the sensory aspect which is important for further use of the gum. As earlier trails have shown: pigs did not seem to dislike the taste of *L. campestre*. The seed gum is most likely tasteless and as tested in this study, even

has a lack of smell which is good for the possible field of use for the gum. For the pectin, it would also be interesting to perform methylation analysis to find out more about the DM of the polysaccharides. This is also important to know when using the seed gum in the food industry, as the DM influence the field of use for the gum.

The seed gum of *L. campestre* has in this study shown to possess many different qualities and functions. As the baking showed, the seed gum has great potential as a thickener and stabilizer in bread, but also in other food products. Because the seed gum is a by-product and easily extracted, the cost is also beneficial for the crop. A small cost is an advantage for *L. campestre* in the market when to compete with other more familiar hydrocolloids e. g. guar gum, garden cress and chia seeds. With the high pectin and DF value in the seed gum, the substance could also be used as a DF enrichment in all kinds of food products for the general health conditions in terms of the daily intake of DF.

6 Conclusion

The seed gum from *L. campestre*, field cress, is consisting of up to 80 % DF, with a total yield of 7 % from weight seeds. The DF in the gel is mostly made of a rhamnogalactouronic pectic substance. The pectin contributes to a very stable and reliable seed gum that could be widely used in the food industry. Possible fields of applications are e. g. production of gluten-free bread or as dietary fibre-enrichment in food products.

In this study, it is also proven that it is a very easily extractable and easily isolated substance that does not have any smell and most likely no taste. From *L. campestre*, it is possible to get a substance made of a up to 80 % pure pectic substance, and all that is required is water and a mixer.

References

- AACC International. (2001). The Definition of Dietary Fiber. *Report of the dietary Fiber definition committee*, 46:3 <http://www.aaccnet.org/initiatives/definitions/Documents/DietaryFiber/DFDef.pdf> [2017-05-03]
- Alfredo, V.O., Gabriel, R.R., Luis, C.G. and David, B.A. (2009). Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica* L.) *LWT - Food Science and Technology*, 42:168–173.
- Andersson, A.A.M., Merker, A., Nilsson, P., Sorensen, H. & Åman, P. (1999). Chemical composition of the potential new oilseed crops *Barbarea vulgaris*, *Barbarea verna* and *Lepidium campestre*. *Journal of Science of Food and Agriculture*, 79:179-186.
- Arefaine, H. (2016). *Lepidium cake as a feed stuff to pigs*. Swedish University of Agricultural Sciences. Department of Animal Nutrition and Management (2016:551)
- Behrouzian, F., Razavi, S.M.A. & Phillips, G.O. (2014). Cress seed (*Lepidium sativum*) mucilage, an overview. *Bioactive Carbohydrates and Dietary Fibre*, 3:17–28.
- Coorey, R., Tjoe, A. & Jayasena, V. (2014). Gelling Properties of Chia Seed and Flour. *Journal of Food Science*, 79(5):859-866.
- Dhingra, D., Michael, M., Rajput, H. & Patil, R.T. (2012). Dietary fibre in foods: a review. *Journal of Food Science Technology*, 49:255-266
- Diwakar, B.T., Dutta, P.K., Lokesh, B. R., & Naidu, K. A. (2008). Bioavailability and metabolism of n-3 fatty acid rich garden cress (*Lepidium sativum*) seed oil in albino rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 78:123–130.
- European Food Safety Authority (2010). *EFSA sets European dietary reference values for nutrient intakes*. Available at: <https://www.efsa.europa.eu/en/press/news/nda100326> [2017-05-23]
- Geleta, M., Zhu, L.H., Stymne, S., Lehrman, A. & Hansson, S.O. (2014). Domestication of *Lepidium campestre* as part of Mistra Biotech, a research programme focused on agro-biotechnology for sustainable food in Batello, C., Wade, L., Cox, S., Pogna, N., Bozzini, A. & Choptiany, J. (Eds.) *Perennial crops for food security. Proceedings of the FAO expert workshop*. Rome: Italy, 28-30 August 2013 (pp. 141-147). FAO, Italy.
- Gokavi, S.S., Malleshi, N.G. & Guo, M. (2004). Chemical Composition of Garden Cress (*Lepidium sativum*) Seeds and Its Fractions and use of Bran as a Functional Ingredient. *Plant Foods of Human Nutrition*, 59:105-111.
- Ivarson, E., Ahlman, A., Li, X. & Zhu, L.H. (2013). Development of an efficient regeneration and transformation method for the new potential oilseed crop *Lepidium campestre*. *BioMed Central Plant Biology*, 13(115).

- Ivarsson, E., Leiva-Eriksson, N., Alhman, A., Kanagarajan, S., Bülow, L. & Zhu, L.H. (2017) Effects of overexpression of *WRI1* and hemoglobin genes on the seed oil content of *Lepidium campestre*. *Frontiers in Plant Science*, 7:2032.
- Joshi, A.A., Bhokre, C.K. & Rodge, A.B. (2015). Evaluation of gum content and viscosity profile of different genotypes of guar from different locations. *International Journal of Current Microbiology and Applied Sciences*, 4(4):553-557.
- Karazhiyan, H., Razavi, S.M.A., Phillips, G.O., Fang Y., Al-Assaf, S., Nishinari, K & Farhoosh, R. (2009). Rheological properties of *Lepidium sativum* seed extract as a function of concentration, temperature and time. *Food Hydrocolloids*, 23: 2062–2068
- Lattimer, J.M. & Haub, M.D. (2010) Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients*, 2010(2):1266-1289.
- Lin, K-Y., Daniel, J.R. & Whistler, R.L. (1994). Structure of chia seed polysaccharide exudate. *Carbohydrate Polymers*, 23:13-18
- Merker, A., Eriksson, D. & Bertholdsson, N.O. (2010). Barley yield increases with undersown *Lepidium campestre*. *Acta Agric. Scand. S. B. Soil & Plant Science*, 60:269-273.
- Mistra Biotech. (2014). *Mistra Biotech Annual Report 2014*. http://www.mistra.org/download/18.5114b7214fae9d7a8aa1cb8/1473225435954/MistraBiotech_AR2014_150301_96dpi_WEB.pdf [2017-05-01]
- Mistra Biotech. (2016) *Mistra Biotech Annual Report 2016*. <https://www.slu.se/globalassets/ew/org/centrb/mbiot/publikationer/mistrabiotech-ar2016-web.pdf> [2017-05-17]
- Mudgil, D. & Barak, S. (2013). Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *International Journal of Biological Macromolecules* 61:1–6
- Mudgil, D., Barak, S., Khatkar, B.S. (2014). Guar gum: processing, properties and food applications—A Review. *Journal of Food Science Technology*, 51(3):409–418.
- Müller, C., Agerbirk, N., Olsen, C.E., Boevé, J.L., Schaffner, U. & Brakefield, P.M. (2001). Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *Journal of Chemical Ecology*, 27:2505-2516.
- Nilsson, P., Johansson, SÅ. & Merker, A. (1998). Variation in seed oil composition of species from the genera *Barbarea* and *Lepidium*. *Acta Agric. Scand. S. B. Soil & Plant Science*, 48:159-164.
- Ramos, S., Fradinho, P., Mata, P. & Raymundo, A. (2016). Assessing gelling properties of chia (*Salvia hispanica* L.) flour through rheological characterization. *Journal of Food and Agriculture*. 97(6):1753–1760.
- Renard, C.M.G.C., Crépeau, M.J. & Thibault, J-F. (1995). Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydrate Research*, 275:155-165.
- Ridley, B.L., O'Neill, M.A. & Mohnen, D. (2001). Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, 57:929–967
- Rosenbohm, C., Lundt, I., Christensen, T.M.I.E. & Young, N.W.G. (2003). Chemically methylated and reduced pectins: preparation, characterization by ¹H NMR spectroscopy, enzymatic degradation, and gelling properties. *Carbohydrate Research*, 338:637-649.
- Sharma, R., Kamboj, S., Khurana, R., Singh, G. & Rana, V. (2015). Physicochemical and functional performance of pectin extracted by QbD approach from *Tamarindus indica* L. pulp. *Journal of Carbohydrate Polymers*, 134:365-374.
- Tamaki, Y., Konishi, T., Fukuta, M. & Tako, M. (2007). Isolation and structural characterisation of pectin from endocarp of *Citrus depressa*. *Journal of Food Chemistry*, 107:352-361.

- Taylor & Francis Group, LLC. (2006). *Food Polysaccharides and Their Applications*. 2. ed. Boca Raton: CRC Press & Taylor & Francis Group. Available at: <https://ttnngmai.files.wordpress.com/2012/10/foodpolysaccharidestheirapplications.pdf> [2017-05-22]
- Theander, O., Åman, P., Westerlund, E., Andersson, R. & Pettersson, D. (1995). Total dietary fiber determined as neutral sugar residues, uronic acid residues, and klason lignin (The Uppsala Method): Collaborative Study. *Journal of AOAC International*, 78(4).
- Thermo Scientific. (2013). *Extinction Coefficients*. Rockford, USA: Thermo Scientific (Tech Tip #6)
- Voragen, F., Beldman, G. and Schols, H. (2000). *Chemistry and Enzymology of Pectins, in Advanced Dietary Fibre Technology*. Oxford, UK: Blackwell Science Ltd. (Chapter 32-33).
- Wennerberg, K., Nylander, T. & Nyman, M. (1991). Rheological and chemical properties of mucilage in different varieties from linseed (*Linum usitatissimum*). *Acta Agric. Scand*, 41:311-319.

Acknowledgement

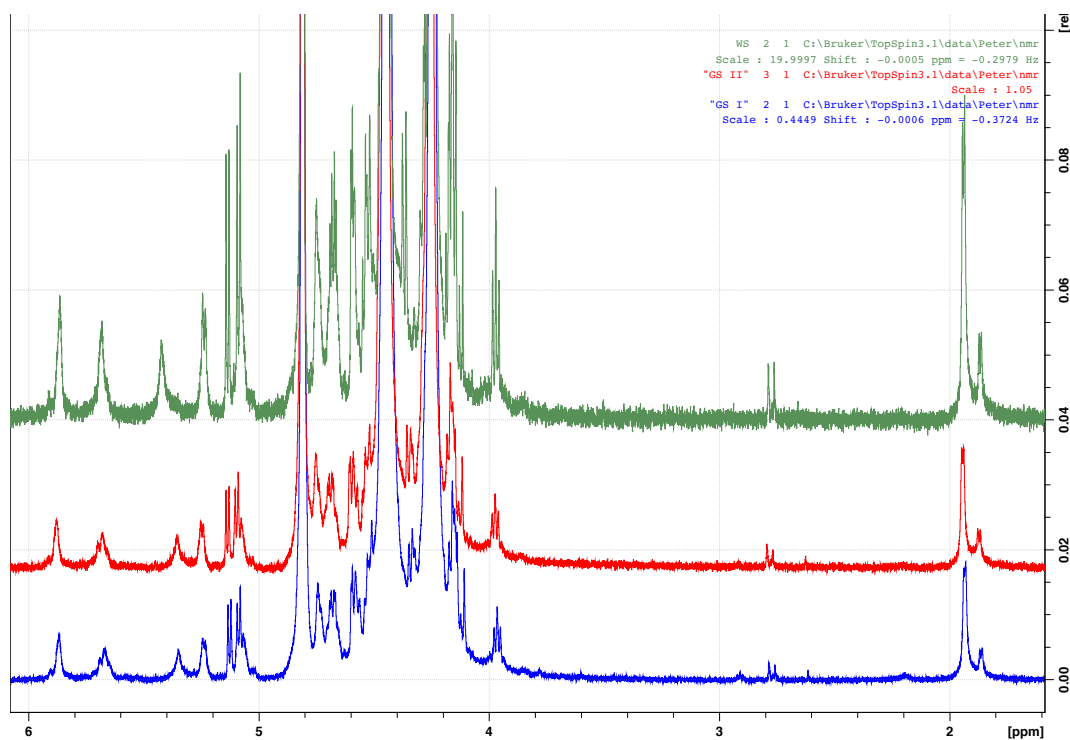
I would like to thank Nils-Ove Bertholdsson at the Department of Plant Breeding at SLU in Alnarp, for providing the seeds used in this study. Thanks to Peter Agback at the Department of Molecular Sciences at SLU in Uppsala for performing the NMR spectroscopy analyses.

Additionally, many thanks to laboratory assistant Gunnel Fransson, for all the time and help with the practical work, especially with the determination of total dietary fibre. You have provided me with a lot of practical knowledge and a new-found confidence in the laboratory.

Finally, I would give a special thanks to my supervisor Roger Andersson at the Department of Molecular Sciences, for all his time, dedication and effort in this project. This thesis has for me come with joy, dedication, hard work and excitement, thanks to you.

Appendix 1: ^1H NMR spectrum

Figure is showing the NMR spectrum from analysis with EDTA treated samples. The broad and big signals between 4-5 is the result of EDTA left in the analysed samples. N. b that the scale is not set in relationship to the water signal and should therefore be adjusted approximately one ppm to the right.



Appendix 2: Popular scientific summary

A pectin rich seed gum from field cress, *Lepidium campestre*

Field cress (*Lepidium campestre*) is a wild species belonging to the *Brassicaceae* family that is distributed all over the world. It is an oilseed species with a great potential of becoming a new commercial crop in Sweden for food, bio-diesel and by-products such as animal feed and packaging material. Because of its great potential, it is part of Mistra Biotech – a program using biotechnology where one of the aims is to domesticate field cress into a novel oil- and cover crop.

As the oil has been extracted from the crop, the residual from the extraction is left in form of a seed cake. This seed cake can possibly be used as livestock feed, but as it is soaked in water, a clear gel is exuded from the seeds mucilage layer, called seed gum, that surrounds the seed. Similar gels extracted from different known seeds are e. g. formed in chia, garden cress guar bean (or cluster bean) and linseed. The purpose of this thesis was to study the chemical composition of the seed gum and to examine if there is a possible field of use for the substance. In this study, there is only one kind of seeds from one harvest analysed and different kinds of varieties within the species *L. campestre* are not studied.

As concluded in the thesis, the seed gum from field cress is mostly consisting of dietary fibre, with a total seed gum yield of 7 % from weight seeds. Dietary fibre is important for many reasons, it is shown to have several positive health effects. A high intake of dietary fibre has a decreased incidence of several types of diseases by lowering cholesterol, regulate blood pressure and by adding bulk to the diet. The dietary fibre in the gel is mostly made of a rhamnogalactouronic pectic substance, a pectin. The pectin contributes to a very stable and reliable seed gum that could be widely used in the food industry. Possible fields of applications are e. g. production of gluten-free bread which also has been successively tested in the study, or as dietary fibre-enrichment in food products.

It is in the study proven, that the seed gum is a very easily extractable and easily isolated pectin rich substance, that does not have any smell and most likely no taste. From the crop field cress, *L. campestre*, it is possible to get a substance made of a up to 80 % pure pectic substance, and all that is required is water and a mixer.